U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, DC 20250-3630

CHAPTER 22

SDI MYCO✓ AFLATOXIN TEST STRIPS

22.1 GENERAL INFORMATION

The Myco✓ Aflatoxin test kit uses lateral flow test strip technology that provides qualitative results for detecting total aflatoxins at a threshold of 20 ppb in corn. Test samples are considered negative when the result is less than 20 ppb (< 20 ppb). Test samples are considered positive when the result is equal to or greater than 20 ppb (≥ 20 ppb).

22.2 PREPARATION OF EXTRACTION SOLUTION

The PBS Buffer for this kit is shipped as a powder and must be mixed with methanol to prepare the 70% Methanol Extraction Solution.

a. PBS Buffer.

(1) Pour the contents of the PBS Buffer packet into a 1-liter carboy or other suitable container.

(2) Add one (1) liter of tap water to the sample buffer and shake until it is completely mixed.

(3) Label the container as PBS Buffer, date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

b. 70% Methanol Extraction Solution.

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean container.

(2) Add 300 ml of PBS Buffer to the methanol and shake until it is completely mixed.

(3) Label the container as 70% Methanol Extraction Solution, the date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed. This solution expires thirty (30) day from the date of preparation.

22.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the 70% Methanol Extraction Solution.

c. Cover the extraction jar and shake by hand for 3 minutes.

d. Allow the sediment to settle for 5 minutes. It is important to not to transfer sediment to the test strip since it can interfere with the flow of liquid and this may affect test results.

NOTE: Sample may also be filtered through Whatman no. 1 filter paper or glass fiber filter provided with the Myco✓ Extraction Kit.

e. It is important to allow the recommended settling time. Test line intensity may increase when testing very fine ground samples if sufficient settling has not occurred.

22.4 TEST PROCEDURES

a. Assay procedure.

(1) Using the calibrated transfer pipette included in the test kit, place 250 microliters (250 µl) of PBS Buffer into a sample tube.

(2) Add 250 µl from the top (yellowish) layer of the extract using a new calibrated transfer pipette to the sample tube.

(3) Insert test strip into the liquid and allow test to develop.

(4) Read the results after 5 minutes and interpret according to Section 22.4.c.

NOTE: To ensure correct volumes are used to prepare the test sample, a calibrated transfer pipette is included with the kit. When a liquid drawn to the top of the straw end of the pipette is dispensed, 250 µl will be expelled into the sample tube. Any overfill is retained in the pipette. Do not reuse diluted samples.

b. Test Strips.

(1) The Myco✓ Aflatoxin Test Strips and Extraction Solution should be stored at room temperature. Do not refrigerate or freeze. Once opened, the strips must be stored in a closed desiccant pack canister. If the moisture-indicating card is pink, contact SDI Technical Service. Storage conditions higher than room temperature may adversely affect performance.

(2) Place the strip into the sample tube containing the diluted sample extract. The arrow tape on the end of the strip should point into the reaction vial.

(3) Test strips should be interpreted after five (5) minutes. Test strips interpreted after ten (10) minutes are invalid.

c. Interpreting the Lateral Flow Test Strip.

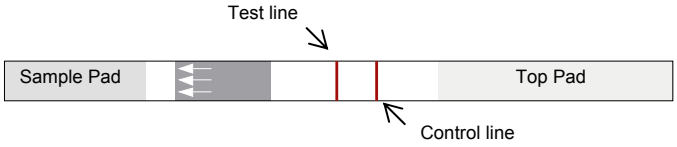
Check the result window at five (5) minutes after inserting the strip. At least one line, the Control Line, should always develop approximately one (1) cm down from the Top Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a negative result. If the test strip displays two (2) red lines, the test is complete and the sample is negative for aflatoxin-contaminated corn. If at 5 minutes the test strip only shows a clearly visible Control Line, then the sample is positive for aflatoxin contamination at or above a 20 ppb level. If no control line develops, the result is inconclusive and needs to be repeated.

(1) Negative Result.

A sample containing aflatoxin residues less than 20 ppb will develop 2 distinct lines, the Control Line and the Test Line, in the test area.

(2) Positive Results.

A sample containing aflatoxin residues equal to or greater than 20 ppb will develop 1 distinct line, the Control Line.



Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	Distinct test line present	< 20 PPB
Control line present	No test line present	20 ppb and higher

22.5 REPORTING AND CERTIFYING TEST RESULTS

Report results on the pan ticket and inspection log as being less than 20 ppb (< 20 PPB), or equal to or greater than 20 ppb (≥ 20 ppb).

Certify results as being less than 20 ppb or equal to or greater than 20 ppb, as applicable.

NOTE: Under the CuSum loading Plan samples reported as 20 ppb or greater will be interpreted as exceeding the 20 ppb threshold and reported accordingly.

Refer to the Aflatoxin Handbook Chapter 4 for detailed certification procedures when using this test kit..

22.6 CLEANING LABWARE

a. Negative Tests (< 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (≥20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard.

22.7 WASTE DISPOSAL

a. Negative Results (< 20 ppb).

If the test result is negative (less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (≥ 20 ppb).

If the result is positive (equal to or greater than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing the remaining filtered extract in the chemical waste container. Pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry.

After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the drain. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

22.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

(1) 50 Myco✓ Aflatoxin Test Strips

(2) 50 sample tubes

(3) 100-250 µl transfer pipettes

(4) 1 Extraction Buffer (Packet)

b. Materials Required but not Provided:

(1) Myco✓ Extraction Kit (7000124) (includes sample cups, and lids, syringe filters, and test tubes)

(2) 100 ml graduated cylinder

(3) Sample grinder

(4) Methanol - Reagent grade or better

(5) Tap water

22.9 STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

Test kits should be stored at room temperature.

b. Precautions.

(1) Do not use the test kits beyond the noted expiration date.

(2) Prolonged exposure to high temperatures may adversely affect the test results.

(3) Do not open the desiccated canister until ready to use the strips.